Title: The "isohydric trap": a proposed feedback between water shortage, stomatal regulation and nutrient acquisition drives differential growth and survival of European pines under climatic dryness

Running head: Climate-induced nutrient imbalance in pines

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Keywords: Climatic change, hotter drought, stable isotopes, nutrients, stoichiometry, stomatal behaviour, water use efficiency

Type of paper: Primary Research Articles

Abstract

Climatic dryness imposes limitations on vascular plant growth by reducing stomatal conductance, thereby decreasing CO_2 uptake and transpiration. Given that transpiration-driven water flow is required for nutrient uptake, climatic stress-induced nutrient deficit could be a key mechanism for decreased plant performance under prolonged drought. We propose the existence of an "isohydric trap", a dryness-induced detrimental feedback leading to nutrient deficit and stoichiometry imbalance in strict isohydric species. We tested this framework in a common garden experiment with 840 individuals of four ecologically-contrasting European pines (*Pinus halepensis*, *P. nigra*, *P. sylvestris*, and *P. uncinata*) at a site with high temperature and low soil water availability. We measured growth, survival, photochemical efficiency, stem water potentials, leaf isotopic composition ($\delta^{13}C$, $\delta^{18}O$), and nutrient concentrations (C, N, P, K, Zn, Cu). After two years, the Mediterranean species *Pinus halepensis* showed lower $\delta^{18}O$ and higher $\delta^{13}C$ values than the other species, indicating higher time-integrated transpiration and water-use efficiency (WUE), along with lower predawn and midday water potentials, higher photochemical efficiency, higher leaf P and K concentrations, more balanced N:P and N:K ratios, and much greater dry-biomass (up to 63-fold) and survival (100%). Conversely, the more mesic mountain pine species showed higher leaf $\delta^{18}O$ and lower $\delta^{13}C$, indicating lower transpiration and WUE, higher water potentials, severe P and K deficiencies and N:P and N:K imbalances, and poorer photochemical efficiency, growth, and survival. These results support our hypothesis that vascular plant species with tight stomatal regulation of transpiration can become trapped in a feedback cycle of nutrient deficit and imbalance that exacerbates the detrimental impacts of climatic dryness on performance. This overlooked feedback mechanism may hamper the ability of isohydric species to respond to ongoing global change, by

aggravating the interactive impacts of stoichiometric imbalance and water stress caused by anthropogenic N deposition and hotter droughts, respectively.

Introduction

Plant survival and performance is determined largely by soil water availability, since water is a major limiting resource for primary production in many regions (Adams et al., 2017; Cramer & Hoffman, 2015; Lawlor & Cornic, 2002). The responses of plant species and individuals to water shortage span a variety of mechanisms that either tend to increase water uptake (e.g. enhanced root growth) or reduce water loss (e.g. stomatal closure). Stomatal regulation is the quickest mechanism to cope with drought, leading to two contrasting strategies termed anisohydry and isohydry, characterized by relaxed vs. tight stomatal control of transpiration in response to decreases in plant water potential (Moran, Lauder, Musser, Stathos, & Shu, 2017; Tardieu & Simonneau, 1998; but see Martínez-Vilalta & Garcia-Forner, 2017). These strategies are clearly the opposite extremes of a continuous ecophysiological gradient (Klein, 2014) but, overall, the degree of stomatal regulation of transpiration is currently considered a key functional trait that explains not only individual plant response to drought, but also forest persistence under current and future climatic conditions (McDowell et al., 2011).

Although the role of stomatal control as a key mechanism in the regulation of plant carbon balance and hydraulic functioning under drought and heat stress has been the focus of much research attention in recent years (Garcia-Forner, Biel, Savé, & Martínez-Vilalta, 2016; Martínez-Vilalta & Garcia-Forner, 2017; McDowell et al., 2013), less attention has been paid to plant nutrient dynamics as a potential key factor influencing plant survival and growth under conditions of prolonged climatic dryness (Gessler, Schaub, & McDowell, 2017; Hartmann et al., 2018). Nutrient availability and

plant nutrient status influence forest productivity through photosynthetic, allocation, and stoichiometric effects (Marschner, Kirkby, & Cakmak, 1996; Sardans & Peñuelas, 2012). Drought decreases soil nutrient availability for plants due to reduced ion mobility and microbial activity, which can lead to impairment of the plant's nutrient status and growth (Kreuzwieser & Gessler, 2010). Plant nutrient acquisition from the soil is tightly linked to water uptake and movement in soils, as plants rapidly deplete nutrients from the rhizosphere, which must be replenished by dissolved nutrients carried in the transpiration-driven mass flow of water to plant roots (Cabrera-Bosquet, Sánchez, & Araus, 2009; Lambers, Chapin, & Pons, 2008; Voltas, Romagosa, Muñoz, & Araus, 1998). Plant nutrient uptake is therefore heavily dependent on the existence of a negative water potential gradient from the soil to the roots that is driven by leaf transpiration (Lambers, Chapin, & Pons, 2008). Furthermore, plants need to maintain particular nutrient stoichiometric relations in their tissues for proper ecophysiological functioning (Güsewell, 2004; Koerselman & Meuleman, 1996; Marschner et al., 1996; Sardans & Peñuelas, 2012). Given that nutrient mobility in the soil matrix may differ by several orders of magnitude among various essential macro- and micronutrients (Lambers et al., 2008), environmental conditions of dryness forcing stomatal closure may severely impair the nutrient balance and stoichiometric ratios of plant tissues. Thus, the interplay and interdependence between plant water relations, nutrient status and stoichiometric relations should be considered a potential important mechanism contributing to plant mortality or reduced performance in models that seek to predict the impact of drier climatic conditions on plant communities.

Here, we propose the existence of an "isohydric trap" that occurs when vascular plant species with a strict isohydric behaviour fall under prolonged climatic dryness conditions, leading to a detrimental feedback loop between water stress, tight stomatal

104 control, and nutrient uptake and status that can be detrimental to plant physiological
105 functioning including growth and survival (Figure 1). In this conceptual model, strict
106 isohydric species exhibit early and prolonged stomatal closure under dry conditions
107 (Klein, 2014; Moran et al., 2017), thereby drastically reducing cumulative transpiration,
108 and hence mass flow of water and in-solution nutrients to roots. As a result, nutrient
109 uptake decreases and plants become prone to macro- and micronutrient deficiency and
110 stoichiometric imbalance, which in turn further decreases stomatal conductance and
111 carbon assimilation through reductions in photochemical efficiency and water use
112 efficiency (Figure 1; blue arrow). These feedbacks eventually decrease carbon
113 availability for supporting root and ectomycorrhizal activity and growth, further
114 reducing the capacity for plant water and nutrient uptake (León-Sánchez et al., 2017)
115 and thereby further impairing the plant's ability to cope with prolonged climatic dryness
116 (Figure 1; green arrow).

117 In this study, we seek to demonstrate that the detrimental impact of this
118 "isohydric trap" on plant nutrient status and stoichiometry is a key mechanism behind
119 the response of drought-sensitive plant species to prolonged climatic dryness. To test
120 this theoretical framework, we conducted a two-year common garden experiment where
121 juveniles of four pine species with contrasting ecological niche and ecophysiological
122 behaviour were grown under the same xeric environmental conditions. Plants were
123 monitored for survival and growth, along with photochemical efficiency, water
124 potential, leaf nutrient concentrations, and leaf $\delta^{18}O$ and $\delta^{13}C$ signatures, which were
125 used as surrogates of time-integrated stomatal conductance and water use efficiency,
126 respectively. We predicted that exposure to prolonged climatic dryness will impose
127 strong constraints on cumulative transpiration and nutrient uptake in pine species with
128 strict isohydric behaviour. Due to the tight coupling between water and nutrient uptake

by roots, reduced transpiration will hamper the mass flow and diffusion of nutrients to roots and the uptake of dissolved nutrients, eventually leading to nutrient deficiency and severe N:P:K stoichiometric imbalance. This will hinder plant carbon balance, transpiration and water use efficiency, ultimately leading to decreased growth and survival. In contrast, drought-tolerant pine species with less strict stomatal control of transpiration will escape this "isohydric trap" by allowing water potential to drop while maintaining greater stomatal aperture and transpiration under prolonged drought stress, which will allow greater nutrient uptake and a more balanced plant nutrient status and stoichiometry. We seek to expand current knowledge on plant responses to increasing frequency of hotter droughts (Allen, Breshears, & McDowell, 2015) by examining the importance of drought-induced nutrient starvation and stoichiometric imbalance, which to-date has been largely overlooked or underestimated in both conceptual and empirical models of plant responses to climate change (Gessler et al., 2017).

Material and methods

Species and plant material

The studied species were *Pinus halepensis* Mill., *P. nigra* Arnold, *P. sylvestris* L., and *P. uncinata* Ram. These species are native in Europe, altogether cover a wide geographic range across the continent (*circa* 30% of its forest area; Köble & Seufert, 2001) and segregate clearly along aridity gradients in the order *P. halepensis* > *P. nigra* > *P. sylvestris* > *P. uncinata*, whereas their resistance to cold stress follows the opposite trend (Fernández-Pérez, Villar-Salvador, Martínez-Vilalta, Toca, & Zavala, 2018; Ruiz de la Torre, 2006; Tapias, Climent, Pardos, & Gil, 2004). *Pinus halepensis* is widely distributed throughout the Mediterranean basin from sea level to 1200 m a.s.l., *P. nigra* from 800 to 2000 m a.s.l. (Mediterranean and Alpine distribution), *P. sylvestris* from

1000 to 2100 m a.s.l. (Boreo-Alpine/Eurosiberian distribution), and *P. uncinata* is distributed from 1400 to 2200 m a.s.l. in the Alps, Pyrenees, and other high Iberian mountains (Richardson 2000; altitudinal ranges for Southern Europe). In general, pines are considered isohydric species compared to other taxa such as oaks or junipers (Meinzer, Woodruff, Marias, Mcculloh, & Sevanto, 2014; Zweifel, Steppe, & Sterck, 2007). However, several evidences support that there is a gradient in stomatal behaviour in response to water stress among the studied species. First, these species show differences in the regulation of plant water potential, which is associated with stomatal control (Klein, 2014; Sperry, Hacke, Oren, & Comstock, 2002; Tardieu & Simonneau, 1998), with *P. halepensis* reaching the lowest water potentials, followed by *P. nigra*, *P. sylvestris* and *P. uncinata* (Choat et al., 2012; Matías, Castro, Villar-Salvador, Quero, & Jump, 2017; Oliet, Planelles, López Arias, & Artero, 2002). Second, leaf-level measurements suggest the existence of large interspecific differences in stomatal sensitivity to low plant water potentials, with *P. halepensis* showing the lowest water potentials at stomatal closure, followed by *P. nigra* and *P. sylvestris* (Martin-StPaul, Delzon, & Cochard, 2017). Finally, previous measurements at whole-plant level in the study site showed that *P. halepensis* exhibits less tight stomatal control and higher transpiration rates under dry conditions than *P. nigra* and *P. sylvestris* (Salazar-Tortosa et al., 2018). Therefore, we may assume that the studied species can be ordered along an iso-anisohydry gradient from *P. uncinata* (most isohydric), *P. sylvestris*, *P. nigra* to *P. halepensis* (most anisohydric).

Seeds of the four species were collected when ripe from certified provenance regions of the Iberian Peninsula (Appendix S1, Table S1). The seeds were stored under cold, dry conditions until sowing. Seeding was done in winter 2012 using 300-mL plastic containers filled with fertilized peat (White 420 F6 Kekkilä**,** Finland; pH 4.7)

containing 0.8-1 kg/m^3 of a slow-release fertilizer NPK 16-10-20. They were initially grown in a greenhouse of the Centro Nacional de Recursos Genéticos Forestales "El Serranillo" (Guadalajara, Spain, 40° 39' 56.14" N, 3° 10' 15.20" W) to avoid frost damage. In mid-May 2012, the seedlings were moved outdoors and cultivated under optimal forest nursery conditions until 15 February 2013, when they were transferred to the common garden site. Nutrient content and isotopic composition at the time of transplanting indicates that seedlings had not been subjected to any water or nutrient stress during the nursery stage (Appendix S1, Table S1).

Study site and experimental design

The common garden experiment was conducted at the "Huerta de La Paloma" farm (37º 10' 03.43" N, 3º 36' 57.80" W; Granada, Southern Spain), a flat (slope ca. 2%), agricultural terrain at 649 m a.s.l. The climate is Mediterranean with hot, dry summers and precipitation concentrated in autumn and spring. The mean annual rainfall is 394±38 L m^2 y^{-1} and the mean annual temperature is 15.3±0.1ºC, with a mean maximum of the hottest month of 35.7±0.2ºC and a mean minimum of the coldest month of -0.1±0.2ºC (period 2006-2015; climatic data from a meteorological station located 1.5 km away at IFAPA Research Field Station). These climatic conditions can be regarded as dry and hot for *P. nigra*, *P. sylvestris* and *P. uncinata* when compared to the prevailing climatic conditions in their native ranges (Christensen, 1987; Enescu, de Rigo, Caudullo, Mauri, & Houston-Durrant, 2016; Houston-Durrant, de Rigo, & Caudullo, 2016), whereas they fall within the optimal ecological range of *P. halepensis* (Mauri, Di Leo, de Rigo, & Caudullo, 2016). The soil is deep with a loamy texture, and average values of 44.8% sand, 41.8% silt, and 13.3% clay, and a soil water content of 13% at wilting point and 33% at field capacity (-1.5 and -0.033 MPa, respectively;

means for the profile down to 1 m deep; no marked horizons in soil profile; analyses done in the *Laboratorio Agroalimentario de la Junta de Andalucía,* Atarfe, Granada, official laboratory for the Regional Agricultural Service). The soil-nutrient content at 0-15 cm depth (N, P and K) showed adequate values for plant growth (Appendix S1, Table S2).

On 15 February 2013, the one-year-old seedlings grown under nursery conditions were transplanted to the common garden site using a randomized-block design. Three blocks of 500 m^2 were located side by side, separated by 2.5 m. In each block, we planted a total of 70 individuals of each species (70 x 3 blocks x 4 species = 840 seedlings in total). Within each block, seedlings were regularly planted at 1.25-m distance from each other, and individuals of each pine species where distributed randomly within the planting scheme. Weeds were removed manually and with a cultivator as needed to prevent competition. We did not find any competition effect from neighbours on either survival or growth ($P > 0.53$ in both cases; Appendix S1, Table S3). The initial size of each seedling (length of the leader shoot and stem-root collar diameter) was measured just after planting as a baseline for aboveground growth estimations (Appendix S1, Table S1). Soil-water content was measured regularly throughout the summers (June-September) at 10, 20, 30, 40, 60, and 100 cm depth using a PR-2/6 Soil Moisture Profile Probe (Delta T, Cambridge, UK). Water content in the soil profile remained above the permanent wilting point throughout the hot, dry (summer) season (Appendix S1, Table S4).

Seedling survival and growth

Survival was monitored eight times from 13 June 2013 to 9 September 2014. Seedling growth was measured non-destructively for all the plants in September 2013 and 2014,

229 considering leader shoot length and stem diameter (increment relative to initial values
230 measured after planting). Stem volume was calculated for each year assuming a conical
231 shape for the stem, with basal diameter given by the average of two perpendicular
232 measurements at the root collar and height given by the maximum height of the leader
233 shoot. Growth patterns for both years were similar and hence only the data from 2014
234 are reported. In the third year (September 2015), height, stem-root collar diameter, and
235 fresh weight were measured in a random subsample of five pines per species and block
236 (*P. uncinata* not included due to small sample size; 45 pines in total). Survival was not
237 monitored in the third growing season of the experiment, given that a destructive
238 harvesting of seedlings was performed the previous year for leaf isotopic and nutrient
239 analyses (see below), and thus the remaining plants might not represent a random
240 sample for this variable.

241

242 *Physiological variables*

243 The effective photochemical quantum yield of photosystem II (Y(II), termed quantum
244 yield from now on), relative electron transport rate (rETR), photochemical quenching
245 (qP), non-photochemical quenching (qN), maximum photochemical efficiency of
246 photosystem II (F_v/F_m), and leaf-water potential (Ψ) were measured for a subsample of
247 nine randomly selected seedlings per species and block in July of 2014. Quantum yield,
248 qP, and qN were measured by means of a portable junior PAM fluorometer (Heinz
249 Walz GmbH Germany), and rETR was calculated by means of the following equation
250 (Schreiber, 2004):

251

252 $rETR = PAR \times ETR\text{-}Factor \times P_{PSII}/P_{PPS} \times Y(II)$,

253

where PAR is the photosynthetically active radiation during the measurements; ETR-Factor is the absorptance of photons by photosynthetic pigments which is considered to be 0.84 as a reasonable match to the average absorptance in the visible range (400-700 nm); P_{PSII}/P_{PPS} is the ratio between the photons absorbed by PS II and photons absorbed by photosynthetic pigments, with a value of 0.5 assuming only linear electron transport, that is, equal transfer rates through PS I and PS II, and comparable photochemical quantum yields of PS I and PS II under strongly light-limiting conditions; and Y(II) is the effective photochemical quantum yield of PS II, as described above.

The F_v/F_m was measured at predawn and midday using a portable fluorometer (FMS2, Hansatech Instruments, UK). Plant water potential (Ψ) was also measured at predawn and midday (except for *P. uncinata* which was only measured at predawn due to the low number of surviving individuals) with a pressure chamber (SKPM 1400, Skye Instruments, UK). Measurements were made in lateral branches in most cases. Photosynthetic fluorescence parameters were always measured between 12:00 and 16:00 h (solar time), except F_v/F_m predawn measurements.

Leaf isotopic composition

We used leaf $\delta^{13}C$ and $\delta^{18}O$ as time-integrated proxy measures of intrinsic water-use efficiency (iWUE, which is the ratio between net photosynthetic rate and stomatal conductance; Farquhar et al., 1989) and stomatal conductance, respectively (Barbour, 2007; Farquhar et al., 2007). Given that all the target pine species had very similar needle size and morphology and were exposed to the same environmental conditions in the common garden (including air temperature, vapour-pressure deficit, and soil-moisture content), we assume that interspecific differences in leaf $\delta^{18}O$ should primarily reflect differences in time-integrated cumulative transpiration derived from species-

specific patterns of stomatal regulation of leaf-gas exchange (Barbour, 2007; Farquhar et al., 2007). Likewise, we assume that interspecific differences in leaf $\delta^{13}C$ should primarily reflect differences in iWUE, rather than differences in irradiance or soil-water availability (as these were the same across species; Dawson et al., 2002; Farquhar et al., 1989).

Both $\delta^{18}O$ and $\delta^{13}C$ were measured on fully expanded leaves harvested in late August 2014 (thus after two growing seasons in the field) from 15 individuals per species and replication block (thus totalling 45 individuals per species); in the case of *P. uncinata* we could only sample 13 individuals due to low survival rate. We harvested pine needles produced during the current year 2014, discarding those from previous year cohorts to minimize potential legacy effects from the nursery. The pine individuals were sampled randomly, although we disregarded those with clear symptoms of decay (close to death) and those previously used to monitor physiological performance in order to avoid any bias due to experimental manipulation (e.g. increased physiological stress after cutting of branches for water-potential measurements in the previous month). After harvesting the needles for isotopic and nutrient analysis, the whole-aboveground biomass of the plant was harvested for dry-biomass production measurement. Samples were oven dried at 60 ºC until constant weight and afterwards the two weights (leaves used for isotopic analysis plus the rest of the plant) of each individual were added together for the statistical analyses of aboveground dry-biomass production.

The subsample of pine needles used for isotopic analyses was finely ground to a fine powder with a ball mill. For leaf $\delta^{13}C$, we used 4 mg weighed in tin capsules. Samples were analysed using an Isoprime isotope ratio mass spectrometer (IRMS; Isoprime Ltd, Cheadle Hulme, Stockport, UK) coupled to a CN elemental analyser (EA;

Eurovector, Pavia, Italy) with continuous flow, at the Department of Biology, University of Copenhagen. For foliar $\delta^{18}O$ analyses, 0.7-0.8 mg were weighed in silver capsules and analysed at the Stable Isotope Facility of the University of California at Davis (USA). A Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) was used following the method described in Kornexl, Gehre, Höfling, & Werner (1999). Leaf samples were decomposed in a glassy carbon reactor at 1400°C to CO and H_2O, and oxygen was analysed as CO. We expressed the isotopic composition of the samples in delta notation as:

$$\delta^{xx} = \left(\frac{R_{samp}}{R_{stand}} - 1\right) * 1000$$

where ^{xx}E is the heavy isotope which is compared to the lighter one; R refers to the molar ratio of the heavy to the light isotope (i.e. ^{13}C:^{12}C or ^{18}O:^{16}O); "samp" refers to the sample; and "stand" refers to an international standard (V-PDB for C and V-SMOW for O). International and internal standards were used for validating the quality and precision of isotopic analyses.

Nutrient analysis

The concentrations of several essential macro- and micronutrients (C, N, P, K, Zn, and Cu) were measured on the same finely ground leaf samples used for isotopic analyses. Foliar C and N concentrations were measured with a CN elemental analyser as described above. Leaf K, P, Cu and Zn concentrations were measured by inductively coupled plasma emission spectrometry using a Perkin-Elmer 5500 ICP.

329 *Data analysis*

330 Analyses were performed using R, version 3.3.2 (R Core Team, 2016). We explored
331 different approaches to control for spurious statistical effects induced by spatial
332 heterogeneity. Note that the number of blocks is too low to consider this variable a
333 random factor in a standard mixed-model approach (random factors need to have at
334 least five levels; Crawley, 2002). Therefore, we controlled for spatial heterogeneity
335 including the number of columns and rows as continuous variables in linear models
336 (Appendix S2). Differences across species for all the variables measured, in any case,
337 followed similar patterns whatever the model used.

338 Differences in seedling survival were estimated with a Cox regression model
339 using the *survival* R package (Therneau, 2015; Therneau & Grambsch, 2000). The rest
340 of response variables (growth, physiological variables, leaf isotopic composition and
341 nutrient concentrations) were analysed with linear models. Significant differences
342 between species were tested using Tukey's test. The stem-volume increment was used
343 as a proxy of overall plant growth because it exhibited a close correlation with both
344 plant height ($P \leq 0.001$; $\rho \geq 0.8$ in all species) and stem diameter ($P \leq 0.001$; $\rho \geq 0.6$ in all
345 species).

346

347 **Results**

348 *Demography*

349 Across species, a total of 627 (74.7%) seedlings survived after two growing seasons.
350 Survival rate differed among species ($P < 2.2e\text{-}16$), with an overall value of 100^{a} %
351 for *P. halepensis*, 92.9^{b} % for *P. nigra*, 80^{c} % for *P. sylvestris*, and 25.8^{d} % for *P.*
352 *uncinata* (different superscript letters indicate significant differences among species, P
353 < 0.05 after Tukey's multiple comparison).

354 Growth (stem-volume increment) after two growing seasons also differed
355 greatly among species ($P < 2.2e-16$), with a much higher value for *P. halepensis*
356 (223.14 ± 11.47^{a} cm^3), followed by *P. nigra* (9.41 ± 0.48^{b} cm^3), *P. sylvestris* (5.06 ± 0.29^{c}
357 cm^3), and *P. uncinata* (2.91 ± 0.43^{d} cm^3). Interspecific differences in growth increased
358 even further after the third growing season (September 2015), with mean stem volume
359 of 3000.56 ± 330.25^{a}, 45.26 ± 5.90^{b}, and 16.50 ± 2.20^{c} cm^3, and mean fresh weight of
360 11723 ± 700^{a}, 322 ± 4^{b}, and 110 ± 14^{c} g for *P. halepensis*, *P. nigra*, and *P. sylvestris*,
361 respectively. *Pinus uncinata* was not sampled in 2015 due to the small number of
362 surviving individuals, but their size was visually the lowest of all the species. See
363 Appendix S1, Table S5 for species mean values of stem-root collar and leader shoot
364 height in both years.
365
366 *Physiological variables*
367 Overall, there were large differences in physiological parameters between *P. halepensis*
368 and the rest of the species, with *P. uncinata* in particular showing the poorest
369 performance under the common garden conditions (Table 1). *Pinus halepensis* showed
370 significantly lower predawn and mid-day stem water potentials than the other species
371 during the summer dry season (Table 1), indicating a more anisohydric behaviour.
372 *Pinus halepensis* also showed the highest values for most photochemical parameters
373 including predawn and midday F_v/F_m, quantum yield, rETR, and photochemical
374 quenching, as well as the lowest values of non-photochemical quenching (although
375 without statistically significant differences from *P. nigra* and/or *P. sylvestris* for the
376 latter two variables). On the contrary, *P. uncinata* showed the lowest values for most of
377 these photochemical parameters, with significant differences from the rest of species for
378 quantum yield and rETR but without significant differences from *P. nigra* and/or *P.*

379 *sylvestris* for the remaining fluorescence variables (Table 1).

380

381 *Leaf isotopic composition and nutrient concentrations*

382 Mean leaf $\delta^{13}C$ values differed significantly among pine species (Table 2), with *P.*
383 *halepensis* showing the highest value (indicative of higher time-integrated water use
384 efficiency), followed by *P. sylvestris, P. nigra* and *P. uncinata.* There were also large
385 differences in mean leaf $\delta^{18}O$ values among species, with *P. halepensis* showing the
386 lowest mean value by far (indicative of higher stomatal conductance and cumulative
387 transpiration), followed by *P. nigra*, *P. sylvestris*, and *P. uncinata* (Table 2).

388 Leaf N concentration differed only slightly among pine species, with values
389 ranging from 12.8 mg g^{-1} for *P. nigra* to 18.3 mg g^{-1} for *P. sylvestris* (Table 2). In
390 contrast, leaf P, K, Cu and Zn concentrations differed sharply among species, with *P.*
391 *halepensis* showing about 2-fold (P), 1.7-fold (K), 1.5-fold (Zn) and 1.8-fold (Cu)
392 higher mean concentrations than the other species (Table 2). As a result, mean foliar
393 N:P and N:K ratios were over 2-fold lower in *P. halepensis* than in the other pine
394 species (Table 2). Leaf C:N ratios were less variable across species and ranged from
395 26.5 in *P. sylvestris* to 35.6 in *P. nigra*.

396 The dry-biomass of pines harvested for isotopic analysis followed the same
397 trend described above for shoot volume and fresh weight after three years: 660.9±32.2
398 for *P. halepensis*, 32.0±2.0 for *P. nigra*, 19.2±1.1 for *P. sylvestris* and 10.4±1.6 g for *P.*
399 *uncinata*.

400

401 *Relationships among plant response variables across and within species*

402 Across pine species, leaf $\delta^{18}O$ was strongly negatively correlated with aboveground
403 biomass and leaf P, K, Cu, and Zn concentrations (Figure 2), while it was positively

404 associated with leaf N and N:P ratio (Figure 3), overall indicating increased nutrient
405 status, stoichiometric balance and growth with increasing time-integrated stomatal
406 conductance and transpiration. Conversely, leaf $\delta^{13}C$ exhibited strong positive
407 correlations with biomass production and leaf P, K, Cu, and Zn concentrations across
408 species, indicating increasing water use efficiency with increasing leaf nutrient status
409 and growth. Leaf $\delta^{13}C$ was negatively associated with N:P ratios, but was unrelated to
410 leaf N concentration across species. Interestingly, leaf $\delta^{13}C$ and $\delta^{18}O$ were negatively
411 associated with each other across all four pine species (ρ = -0.48; P = 1.70E-09), which
412 suggests that interspecific variation in $\delta^{13}C$ was primarily driven by variation in
413 photosynthesis (rather than in stomatal conductance).
414 Similar correlation patterns were found also at the within-species level for *P.*
415 *nigra* and *P. sylvestris*: aboveground biomass and stem volume increment were
416 positively associated with leaf P concentration and negatively associated with leaf $\delta^{18}O$,
417 N, and N:P in at least one of the two species (Appendix S1, Figure S1). Similarly, leaf
418 $\delta^{13}C$ was positively correlated with leaf P and Cu concentrations and negatively
419 associated with N:P ratio. Leaf $\delta^{18}O$ was negatively associated with leaf P, Cu, and Zn
420 concentrations and positively correlated with N:P ratio in one or the two species
421 (Appendix S1, Figure S2).
422 Plant water potentials and photochemical fluorescence variables were also
423 significantly correlated with leaf nutrients and isotopes across species. Mean midday
424 water potential was negatively associated with mean leaf K concentration
425 (ρ = –0.991; P = 0.0088). Mean leaf Zn concentration was positively associated with
426 mean quantum yield (ρ = 0.968; P = 0.032) and predawn F_v/F_m (ρ = 0.994; P = 0.0063),
427 while it was negatively associated with qN (ρ = –0.984; P = 0.016), which overall
428 suggests increased photochemical performance with increased leaf Zn status across

429 species. In addition, mean leaf $\delta^{13}C$ was positively correlated with mean quantum yield
430 and predawn F_v/F_m, and was negatively associated with qN (Figure 5), which suggests
431 increased water use efficiency with increased photochemical efficiency across species.
432 Finally, mean leaf $\delta^{18}O$ was negatively associated with mean quantum yield, predawn
433 F_v/F_m and rETR (and positively with mean qN), thus revealing an improved
434 photochemical efficiency with increasing stomatal conductance and cumulative
435 transpiration across species (Figure 6).
436

437 **Discussion**

438 We found that the thermophilous, drought-tolerant pine species *P. halepensis* exhibited
439 100% survival and far greater growth than the other more drought-sensitive mountain
440 pine species evaluated in the common garden experiment. Unsurprisingly, pine species
441 originating from wetter and cooler habitats such as *P. uncinata* and, to a lesser extent, *P.*
442 *sylvestris*, showed the poorest growth and survival under the xeric common garden
443 conditions. These results are expected according to the contrasting ecological
444 requirements of each species and the relatively warm and dry climatic conditions at the
445 study site. However, our study provides insights into the physiological mechanisms
446 underlying the contrasting responses among pine species, and supports the contention
447 that the tight stomatal regulation typical of drought-sensitive mountain pine species
448 makes them fall into an "isohydric trap", in which high stomatal sensitivity to soil water
449 shortage and high atmospheric evaporative demand trigger stomatal closure and lead to
450 a detrimental feedback loop that eventually causes severe nutrient starvation and
451 stoichiometric imbalance under prolonged dry conditions (Figure 1).

452 The large interspecific differences in leaf isotopic ratios and stem water potential
453 indicate that pine species differ in stomatal control under prolonged climatic dryness.

454 According to the dual isotope conceptual model (Grams, Kozovits, Häberle, Matyssek,
455 & Dawson, 2007; Scheidegger, Saurer, Bahn, & Siegwolf, 2000), the combination of
456 high $\delta^{18}O$ and low $\delta^{13}C$ values in the drought-sensitive mountain pine species
457 (compared to *P. halepensis*) indicates low time-integrated stomatal conductance and
458 water use efficiency, as well as low photosynthesis rates (Querejeta, Allen, Caravaca, &
459 Roldán, 2006), which is consistent with the poor growth of these species. This
460 interpretation of isotope data is in strong agreement with gas exchange measurements
461 conducted at whole plant level with transient-state closed chambers in the same
462 common garden experiment, which showed higher stomatal conductance and
463 transpiration, photosynthetic rates and water use efficiency in *P. halepensis* than in the
464 other pine species during the dry season (Salazar-Tortosa et al., 2018). Furthermore, the
465 potential influence of the use of different water sources among species can be discarded
466 as the lower predawn water potential of *P. halepensis* compared to other species would
467 be incompatible with the alternative explanation that it was using a more $\delta^{18}O$ depleted
468 source water stored in deeper, wetter soil layers (Nardini et al., 2016; Voltas,
469 Lucabaugh, Chambel, & Ferrio, 2015; West et al., 2012).

470 The combination of high predawn and midday stem water potentials at the peak
471 of the dry season with low stomatal conductance and cumulative transpiration (inferred
472 from high leaf $\delta^{18}O$ values) indicates a typical isohydric behaviour (Martínez-Vilalta &
473 Garcia-Forner, 2017) in *P. nigra, P. sylvestris* and *P. uncinata.* Under low water
474 availability and high temperature and evaporative demand conditions, plants with
475 isohydric behaviour close their stomata early to maintain relatively high water potentials
476 and prevent dehydration (Klein, 2014; McDowell et al., 2008; Tardieu & Simonneau,
477 1998). Ultimately, this reduction of cumulative transpiration negatively affects the
478 plant's nutrient status given the importance of active transpiration flux along the soil-

479 plant-air continuum for effective soil nutrient uptake in drying soil (Cramer et al., 2009;
480 Lambers et al., 2008; Rouphael et al., 2012; Sardans, Peñuelas, Prieto, & Estiarte, 2008;
481 Sardans, Peñuelas, Coll, Vayreda, & Rivas-Ubach, 2012). The strong negative
482 correlations between leaf $\delta^{18}O$ (proxy of stomatal conductance) and foliar P, K, Cu, Zn
483 concentrations and positive correlation of leaf $\delta^{18}O$ with N:P and N:K ratios across
484 species (as well as within species for P and N:P; Figure 3, Appendix S1 Figure S2)
485 indicate that soil nutrient uptake was severely constrained by low cumulative
486 transpiration in the drought-sensitive mountain pine species under prolonged dryness,
487 relative to *P. halepensis*.

488 Stomatal conductance and transpiration will determine the rate of water
489 extraction from soil, so that species with higher transpiration rates will deplete
490 rhizosphere soil water faster and will hence experience greater reductions in predawn
491 water potentials during dry periods (Martínez-Vilalta & Garcia-Forner, 2017), as found
492 in *P. halepensis*. This ability to allow water potentials to drop while sustaining high
493 stomatal aperture and conductance (i.e. a more anisohydric strategy) may have enabled
494 *P. halepensis* to continue extracting water and dissolved nutrients from soil during
495 prolonged dry periods. A recent study has also reported strong negative correlations
496 between leaf $\delta^{18}O$ and foliar nutrient concentrations across contrasting plant species
497 exposed to the same environmental conditions, suggesting a heavy dependence of plant
498 nutrient uptake and status on stomatal conductance and cumulative transpiration (Prieto,
499 Querejeta, Segrestin, Volaire, & Roumet, 2017). Nitrogen, by contrast, did not seem to
500 be involved in the observed pattern, as this nutrient was negatively associated with
501 biomass and positively associated with $\delta^{18}O$ (Figure 2, 3, Appendix S1 Figure S1),
502 which suggests that the interspecific differences in leaf N concentration were mainly a
503 consequence of differential growth dilution effects (Sabaté & Gracia, 1994) and not of

stomatal behaviour. In addition, the species with lower growth (*P. sylvestris* and *P. uncinata*) could have constitutively high leaf N as supported by higher N concentration for *P. uncinata* at nursery, and by previous studies for *P. sylvestris* (Sardans et al., 2011).

The unusually high leaf N:P and N:K ratios found in the more drought sensitive, mountain pine species are indicative of strong P and K limitation of growth (Güsewell, 2004; Lawniczak, Güsewell, & Verhoeven, 2009; See Table 2 for a comparison with reference values of each species). The severe nutrient deficit and imbalance observed in these drought-sensitive pine species appeared to be a direct consequence of climatic drought stress, as seedling nutrient status was optimal at the beginning of the experiment across species (Appendix S1, Table S1), and given that the soil at the common garden site was not deficient in any nutrient (Appendix S1, Table S2). Indeed, soil nutrient availability in this fertile agricultural field was sufficient to support a balanced leaf stoichiometry with optimal N:P ratios and vigorous biomass growth in *P. halepensis*. Despite much larger growth-dilution effects, leaf P, K, Zn and Cu concentrations were higher and N:P and N:K ratios were much lower in *P. halepensis* than in the other species, indicating that severe nutrient starvation and stoichiometric imbalance in the other species was largely the result of climatic stress, rather than the result of low soil nutrient availability "per se". Deficiency of P, K, Zn, and Cu and severe N:P:K stoichiometric imbalance can impair photosynthesis, transpiration (Figure 1; blue arrow), water-use efficiency and growth, as reported in this study (see also Güsewell, 2004; Sardans & Peñuelas, 2015; Talbott & Zeiger, 1996). This idea is supported by the strong correlations of leaf nutrient concentrations and stoichiometric ratios with both leaf $\delta^{18}O$ and $\delta^{13}C$ ratios and with aboveground biomass across and within species (Figures 2, 3, 4, Appendix S1, Figures S1, S2). We also found a negative

529 association between leaf K concentration and water potential across species, which may
530 be explained by the major role that K plays in drought tolerance (Rivas-Ubach, Sardans,
531 Perez-Trujillo, Estiarte, & Penuelas, 2012; Sardans & Peñuelas, 2015).
532 The rather extreme N:P:K stoichiometric imbalance observed in the drought-
533 sensitive mountain pine species may be explained by differences in mobility and
534 availability in the soil solution among nutrients, given that PO_4^{--} and K^+ have diffusion
535 coefficients that are lower by orders of magnitude than that of NO_3^- (Lambers et al.,
536 2008; Marschner & Rengel, 2012). Our study suggests that uptake of nutrients with
537 limited mobility and diffusion rates in soil (e.g. phosphate, potassium, zinc, copper)
538 may be particularly vulnerable to severe decreases in transpiration and mass flow to
539 roots, compared to highly mobile nutrients like nitrate (Rengel & Marschner, 2005).
540 Besides, atmospheric deposition is several orders of magnitude higher for N than for
541 other nutrients (Peñuelas, Sardans, Rivas-Ubach, & Janssens, 2012), which may also
542 lead to higher foliar N uptake. Therefore, in a global scenario of climate warming
543 combined with increasing rates of anthropogenic N deposition (Güsewell, 2004; Jonard
544 et al., 2015) we should expect plant P status (along with K and micronutrients like Cu or
545 Zn) to be particularly vulnerable to decreases in transpiration fluxes during prolonged
546 periods of climatic dryness, whereas plant N status may be less responsive. In addition,
547 the reduced carbon assimilation exhibited by drought-sensitive mountain pine species at
548 the common garden site (Salazar-Tortosa et al., 2018) could lead to low carbon
549 availability to support the growth and activity of fine roots and ectomycorrhizal (EMF)
550 fungi (Gessler et al., 2017; Matías et al., 2017; Moran et al., 2017). This could hamper
551 even more the assimilation of low mobility nutrients, whose absorption has high energy
552 and carbon costs such as the production of extramatrical EMF mycelium, the secretion
553 of phosphatases and organic acids by roots and mycorrhizae for solubilisation and

554 mineralization of inorganic and organic P, or rhizosphere priming effects (Achat,
555 Augusto, Gallet-Budynek, & Loustau, 2016; Kreuzwieser & Gessler, 2010). Moreover,
556 limited carbohydrate availability and transfer to roots could also constrain the supply of
557 energy and carbon skeletons for nutrient assimilation (Kreuzwieser & Gessler, 2010).
558 Soil moisture content remained above the permanent wilting point throughout
559 the summer dry period in both years of the experiment (Appendix S1, Table S4), which
560 suggests that high temperature and evaporative demand may have also been key drivers
561 of the contrasting responses observed among pine species (McDowell et al., 2015;
562 McDowell & Allen, 2015; Salazar-Tortosa et al., 2018; Williams et al., 2013). In fact,
563 mean summer temperature at the common garden site was considerably higher than that
564 experienced by the mountain pine species in their original habitat (*P. nigra*, *P.*
565 *sylvestris*, and *P. uncinata*). Heat stress can limit stomatal conductance, as plants close
566 their stomata to prevent excessive transpiration and water loss when atmospheric
567 demand for water increases with rising temperatures (Novick et al., 2016; Urban,
568 Ingwers, McGuire, & Teskey, 2017; Zhang, Wollenweber, Jiang, Liu, & Zhao, 2008).
569 Stomatal closure in response to heat and drought stress reduces evaporative leaf cooling
570 (Cook, Dixon, & Leopold, 1964), which may favour heat-induced damage of the
571 photosynthetic machinery that decreases photochemical efficiency (Sharkey, 2005).
572 Lower predawn F_v/F_m values as well as larger predawn F_v/F_m vs. midday F_v/F_m
573 differences in mountain pine species (compared to *P. halepensis)* support the idea that
574 heat stress in combination with high light levels led to both chronic photoinhibition as
575 well as reversible reduced photochemical efficiency during the hottest time of the day in
576 summer (Table 1). Moreover, the detrimental impacts of the combination of high
577 temperatures and drought stress on leaf photochemical efficiency and carboxylation
578 capacity were likely compounded by the effects of macro- and micronutrient deficiency

579 and stoichiometric imbalance in mountain pine species (Cakmak, 2005; Casimiro,
580 Barroso, & Pais, 1990; Eller, Jensen, & Reisdorff, 2016), which may explain the
581 association of low photochemical efficiency (as indicated by low rETR, quantum yield
582 and predawn F_v/F_m and high qN) with low cumulative transpiration (high $\delta^{18}O$) and
583 water use efficiency (low $\delta^{13}C$) across species (Figures 5, 6). Overall, the results of this
584 study suggest that intensified drought stress from higher air temperatures —the "hotter
585 drought" effect (Allen et al., 2015)— might lead to severe nutrient deficit,
586 stoichiometric imbalance, and photosynthetic impairment mediated by reduced
587 cumulative transpiration in drought-sensitive species.

588 In summary, we found that the degree of stomatal regulation of transpiration is
589 tightly linked to plant nutrient status and stoichiometry under dry conditions, and that
590 reduced nutrient uptake is a key mechanism to consider when assessing poor plant
591 growth and survival under prolonged climatic dryness. We propose the existence of a
592 detrimental feedback loop leading to severe P and K starvation and N:P:K imbalance in
593 strictly isohydric vascular plants undergoing prolonged drought stress, such as some of
594 our study pine species. These drought-sensitive species close their stomata at relatively
595 high soil and plant water potentials, and thus are not able to maintain adequate
596 transpiration and nutrient uptake during prolonged drought periods, which leads to
597 severe nutrient imbalance that might exacerbate and accelerate the onset of carbon
598 starvation, hydraulic failure, phloem dysfunction, and their multiple interplays
599 (McDowell et al., 2011; Sala, Piper, & Hoch, 2010; Sevanto, McDowell, Dickman,
600 Pangle, & Pockman, 2014; Zwieniecki & Holbrook, 2009). In contrast, relatively
601 drought-tolerant plant species with a more anisohydric behaviour such as *P. halepensis*
602 can maintain open stomata and transpiration at comparatively lower soil and plant water
603 potentials, thereby escaping this detrimental feedback and avoiding nutrient deficit and

604 imbalance. This study highlights the intimate interplay and interdependence between
605 stomatal regulation, transpiration, carbon assimilation and nutrient status in the response
606 of vascular plants to long periods of climatic dryness (Gessler et al., 2017). We
607 advocate consideration of this proposed conceptual framework (Fig. 1) in order to better
608 understand and predict the impacts of ongoing global change on the performance and
609 survival of pines and other plant species with tight stomatal regulation and strict
610 isohydric behaviour, with special attention to the role of macronutrients with low
611 mobility in soil, such as P and K.
612

613 **Acknowledgements**

614 This work was supported by the projects ECOLPIN (AGL2011-24296), CGL2013-
615 48753-R, the network REMEDINAL 3 (S2013/MAE-2719) of the CAM, by FPU
616 (FPU13/03410) and Juan de la Cierva (FPDI-2013-15867) fellowships from the Spanish
617 Ministry of Education, Culture and Sport to DS and LM, respectively, and by a grant
618 from IES Abroad. We are also grateful to the Centro Nacional de Recursos Genéticos
619 Forestales 'El Serranillo' (MAGRAMA) for cultivating the plants during the nursery
620 stage. Craig D. Allen and five anonymous reviewers provided insightful comments that
621 improved the manuscript.

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Tables

Table 1 Mean values ± SE of physiological variables measured in the four target pine species during mid-summer (dry season) of the second year after planting. F and P values of the "species" factor are shown for each variable. Significant p values are indicated in bold, while differences between species ($P < 0.05$ after Tukey's multiple comparison) are shown with different superscripts.

		Species					
Variables	Sample size	*P. halepensis*	*P. nigra*	*P. sylvestris*	*P. uncinata*	F	Pr(>F)
Predawn Water Potential (MPa)	71	-1.26±0.05^{b}	-0.86±0.03^{a}	-0.87±0.04^{a}	-	43.9	**2.63E-11**
Midday Water Potential (MPa)	131	-2.37±0.04^{b}	-1.88±0.05^{a}	-1.84±0.04^{a}	-1.92±0.04^{a}	30.6	**6.46E-14**
Predawn fluorescence (F_v/F_m)	132	0.8709±0.0024^{a}	0.839±0.005^{b}	0.836±0.006^{b}	0.825±0.006^{b}	17.2	**5.17E-09**
Midday fluorescence (F_v/F_m)	132	0.796±0.007^{a}	0.685±0.017^{bc}	0.721±0.017^{b}	0.657±0.022^{c}	16.3	**1.34E-08**
Yield	127	0.688±0.005^{a}	0.599±0.017^{b}	0.598±0.017^{b}	0.545±0.024^{b}	22.1	**7.35E-11**
rETR (μM e/m^{2}s)	127	455.0±10.0^{a}	396.1±13.3^{b}	384.9±13.7^{b}	324.4±15.9^{c}	17.7	**3.85E-09**
Photochemical quenching	125	0.972±0.003^{a}	0.968±0.004^{ab}	0.958±0.005^{ab}	0.953±0.005^{b}	3.6	**1.66E-02**
Non-Photochemical quenching	126	0.044±0.006^{b}	0.067±0.010^{ab}	0.071±0.009^{ab}	0.083±0.013^{a}	3.2	**2.53E-02**

Table 2 Mean values ± SE of leaf isotopic composition, nutrient concentrations and stoichiometric ratios measured in the four target pine species in mid-summer (dry season) of the second year after planting. F and P values of the "species" factor are shown for each variable. Significant p-values are indicated in bold, while differences between species ($P < 0.05$ after Tukey's multiple comparison) are shown with different superscripts. As a reference, macronutrient concentration and stoichiometric ratios observed in natural populations of the studied species in Northeast of the Iberian Peninsula are shown in brackets (data obtained from Sardans, Rivas-Ubach, & Peñuelas, 2011).

		Species					
Variables	Sample size	*P. halepensis*	*P. nigra*	*P. sylvestris*	*P. uncinata*	F	Pr(>F)
Foliar $\delta^{13}C$ (‰)	186	-25.50 ± 0.09^{a}	-27.68 ± 0.17^{b}	-27.50 ± 0.16^{b}	-28.38 ± 0.32^{b}	60.8	**< 2.2E-16**
Foliar $\delta^{18}O$ (‰)	186	26.72 ± 0.11^{d}	28.59 ± 0.17^{c}	29.55 ± 0.18^{b}	30.12 ± 0.3^{a}	97.3	**< 2.2E-16**
Nitrogen (mg g^{-1})	181	14.3 ± 0.3^{b} (10.6±0.1)	12.8 ± 0.3^{c} (9.4±0.09)	18.3 ± 0.7^{a} (11.9±0.1)	16.5 ± 0.8^{ab} (9.7±0.15)	26.8	**1.06E-13**
Phosphorus (mg g^{-1})	185	0.85 ± 0.03^{a} (0.80±0.01)	0.37 ± 0.02^{c} (0.83±0.01)	0.43 ± 0.02^{b} (1.10±0.01)	0.49 ± 0.03^{bc} 0.92±0.01)	122	**< 2.2E-16**
Potassium (mg g^{-1})	186	4.74 ± 0.14^{a} (4.00±0.05)	2.95 ± 0.10^{b} (4.44±0.08)	2.57 ± 0.12^{c} (5.55±0.09)	2.83 ± 0.21^{bc} (4.16±0.10)	58.4	**< 2E-16**
Zinc (ppm)	185	18.8 ± 0.5^{a}	13.6 ± 0.8^{b}	12.3 ± 0.7^{b}	11.3 ± 1.3^{b}	19.1	**2.17E-10**
Copper (ppm)	184	3.09 ± 0.08^{a}	1.53 ± 0.07^{b}	1.69 ± 0.08^{b}	1.95 ± 0.20^{b}	74.2	**< 2.2E-16**
C:N ratio	186	31.4 ± 0.7^{b} (52.2±0.5)	35.62 ± 0.81^{a} (57.3±0.6)	26.5 ± 0.7^{c} (45.0±0.3)	27.96 ± 1.16^{bc} (55.6±0.8)	27	**8.56E-14**
N:P ratio	180	17.5 ± 0.6^{c} (14.9±1.1)	36.6 ± 1.5^{b} (11.8±0.2)	46.4 ± 2.7^{a} (11.4±0.1)	35.4 ± 2.7^{ab} (10.5±0.2)	99	**< 2.2E-16**
N:K ratio	181	3.13 ± 0.12^{c} (3.00±0.07)	4.59 ± 0.20^{b} (2.55±0.07)	7.84 ± 0.50^{a} (2.63±0.07)	6.20 ± 0.50^{a} (2.70±0.12)	62.1	**< 2.2E-16**

965 *Figure captions*

966 Figure 1 Proposed "isohydric trap" conceptual model of reduced plant physiological
967 performance and growth under hotter drought, in which vascular plants with a strong
968 stomatal regulation of transpiration undergo early and prolonged closure of the stomata
969 under heat and drought stress. This strict isohydric behaviour triggers a series of
970 physiological changes that culminate in severe nutrient imbalance and a reduction of the
971 capacity to cope with further drought stress, thereby becoming a feedback to in earlier
972 stomatal closure under a subsequent event of water stress (the "isohydric trap"). Sub-
973 loops are shown with arrows of different colours: i) Transpiration-nutrient loop in blue;
974 ii) Transpiration-root growth loop in green.

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976 Figure 2 Spearman's rank coefficients (ρ) for the correlations of pine sapling growth
977 after two years with leaf $\delta^{18}O$, $\delta^{13}C$ and nutrient concentration across species.
978 Significant associations are indicated with asterisks (* for $P < 0.05$ and ** for $P < 0.01$).

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980 Figure 3 Correlations between leaf nutrient concentrations and foliar $\delta^{18}O$ (proxy of
981 stomatal conductance) across species. Spearman's rank correlation coefficients along
982 with p and S value are shown for each correlation. Abbreviations: H = *P. halepensis*, N
983 = *P. nigra*, S = *P. sylvestris*, U = *P. uncinata*.

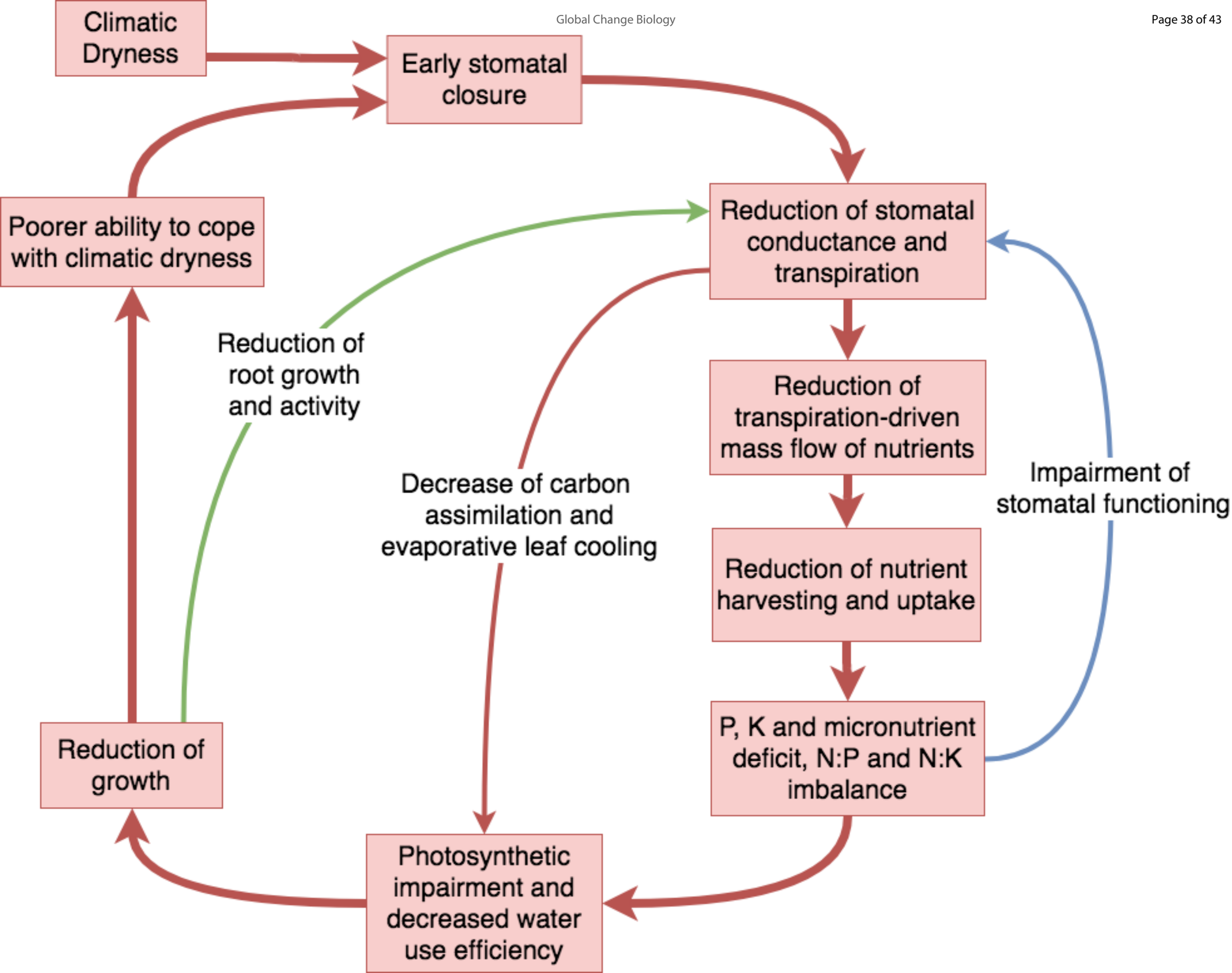
984

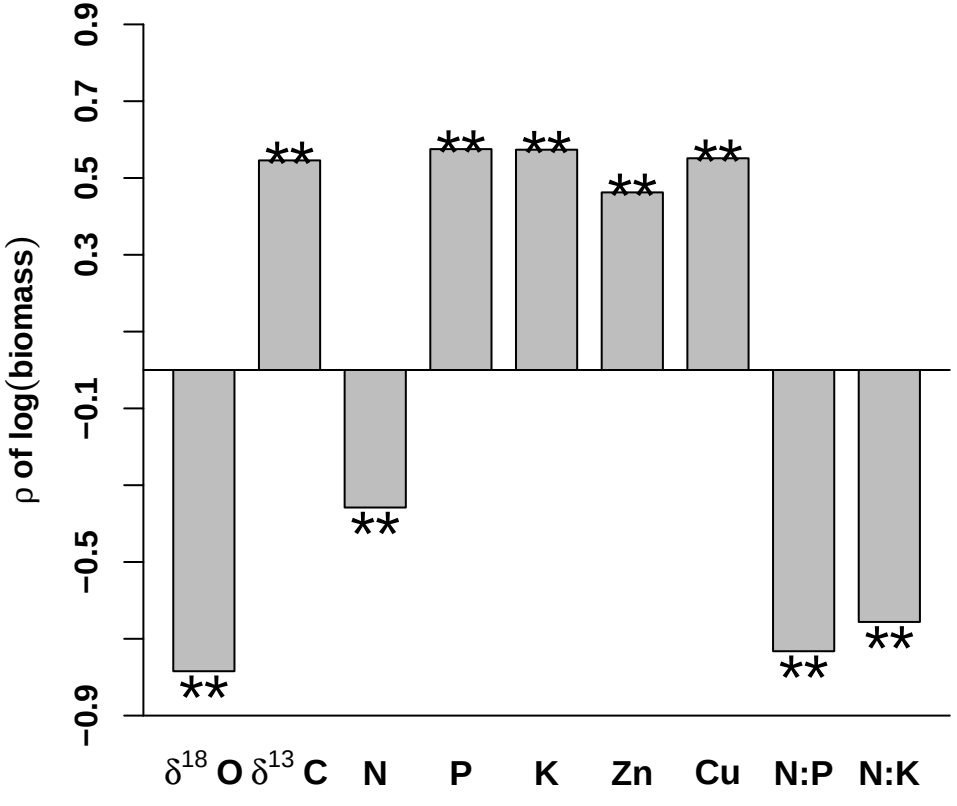
985 Figure 4 Correlations between foliar $\delta^{13}C$ (proxy of water-use efficiency) and leaf
986 nutrient concentrations across pine species. Spearman's rank correlation

coefficients together with p and S value are shown for each correlation. Species abbreviations as in Figure 3.

Figure 5 Correlation between mean values per species of leaf $\delta^{13}C$ (proxy of water-use efficiency) with photosynthetic fluorescence parameters across pine species. Pearson's correlation coefficients along with p and t value are shown for each correlation. Error bars for both axes represent ±SE. Species abbreviations as in Figure 3. Variables abbreviations: Yield = Quantum yield, qN = Non-photochemical quenching.

Figure 6 Correlation between mean values per species of leaf $\delta^{18}O$ (proxy of stomatal conductance) with photosynthetic fluorescence parameters across pine species. Pearson's correlation coefficients together with p and t value are shown for each correlation. Error bars for both axes represent ±SE. Species abbreviations as in Figure 3. Variables abbreviations: Yield = Quantum yield, rETR = relative electron transport rate, qN = Non-photochemical quenching.


Climatic Dryness
Early stomatal closure
Poorer ability to cope with climatic dryness
Reduction of stomatal conductance and transpiration
Reduction of root growth and activity
Decrease of carbon assimilation and evaporative leaf cooling
Reduction of transpiration-driven mass flow of nutrients
Impairment of stomatal functioning
Reduction of nutrient harvesting and uptake
P, K and micronutrient deficit, N:P and N:K imbalance
Reduction of growth
Photosynthetic impairment and decreased water use efficiency


ρ of log(biomass)
0.9
0.7
0.5
0.3
−0.1
−0.5
−0.9
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δ18 O
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N
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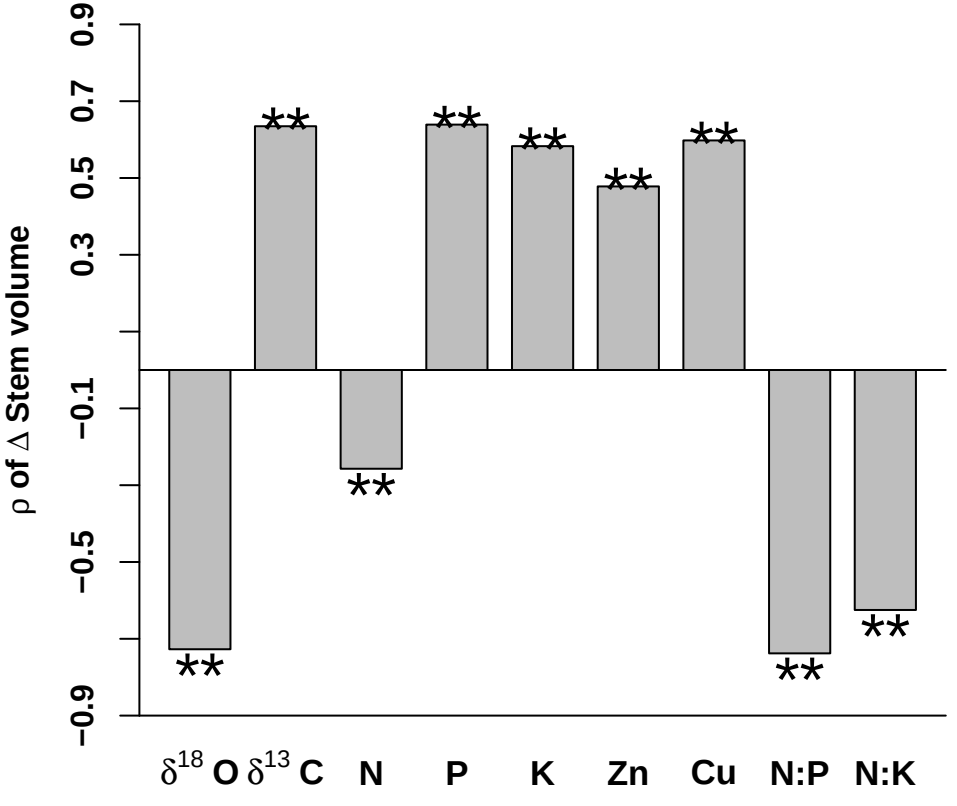
K
Zn
Cu
N:P
N:K


ρ of Δ Stem volume
0.9
0.7
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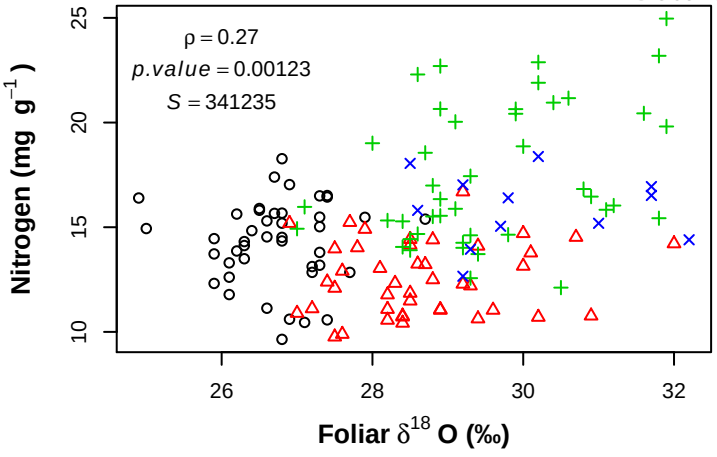
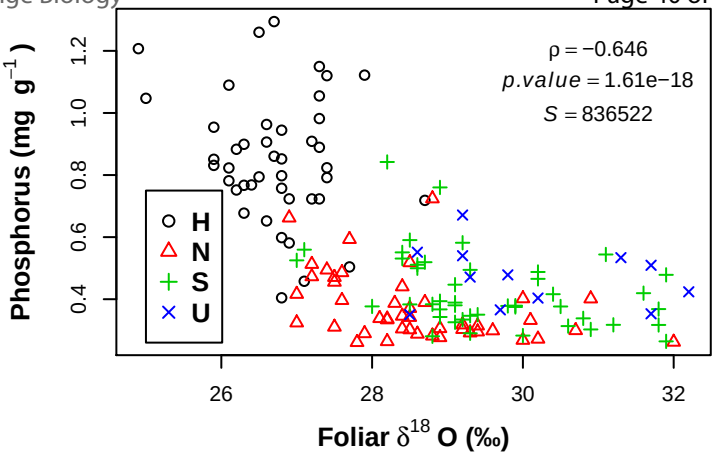
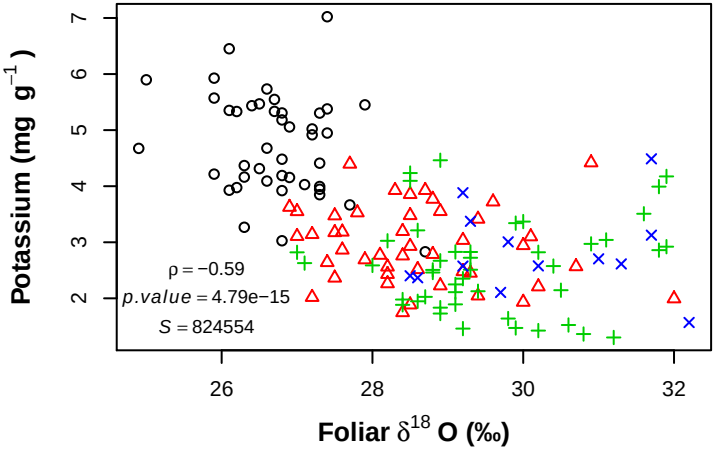
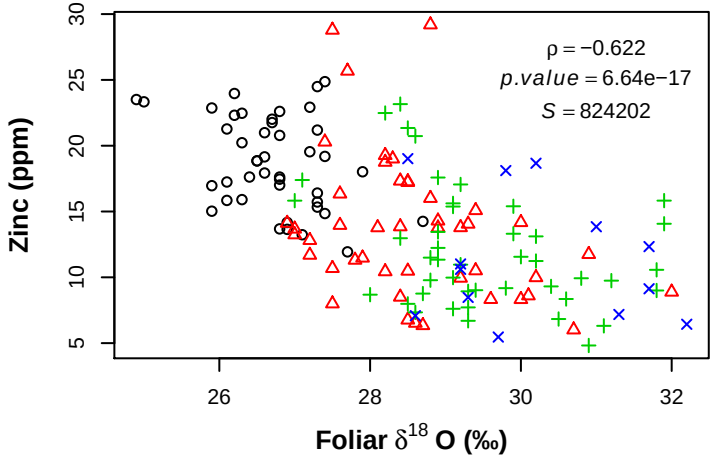
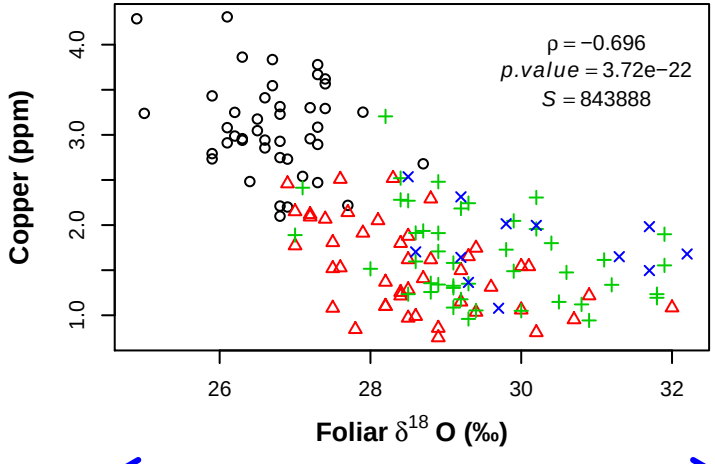
N:P
N:K

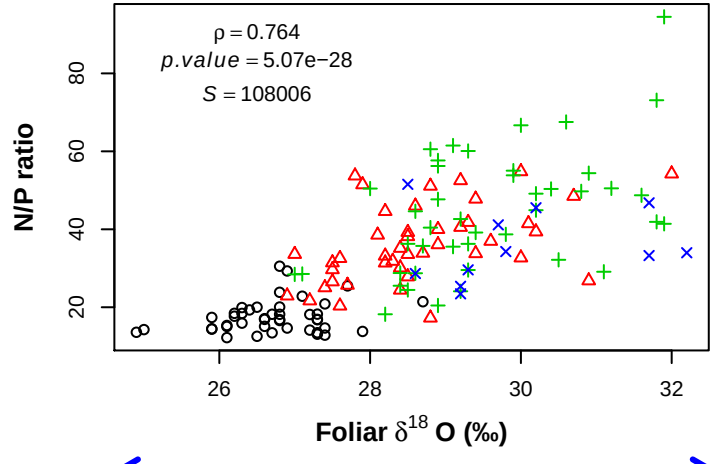

ρ = 0.27
p.value = 0.00123
S = 341235
Nitrogen (mg g^{-1})
Foliar δ^{18} O (‰)

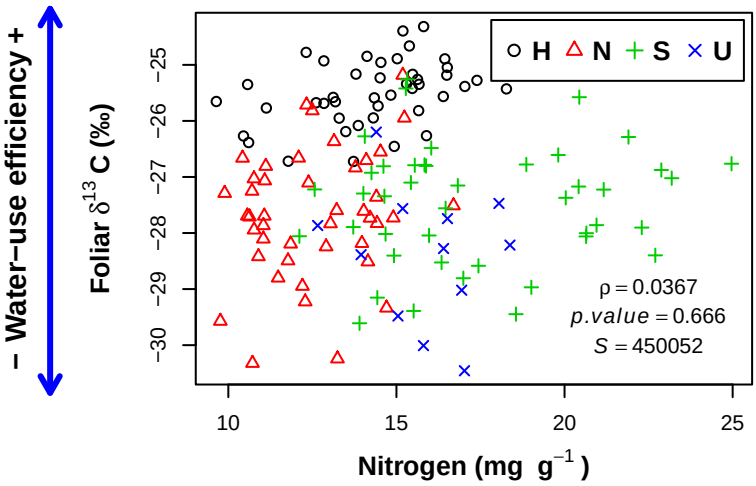

ρ = −0.646
p.value = 1.61e−18
S = 836522
H
N
S
U
Phosphorus (mg g^{-1})
Foliar δ^{18} O (‰)

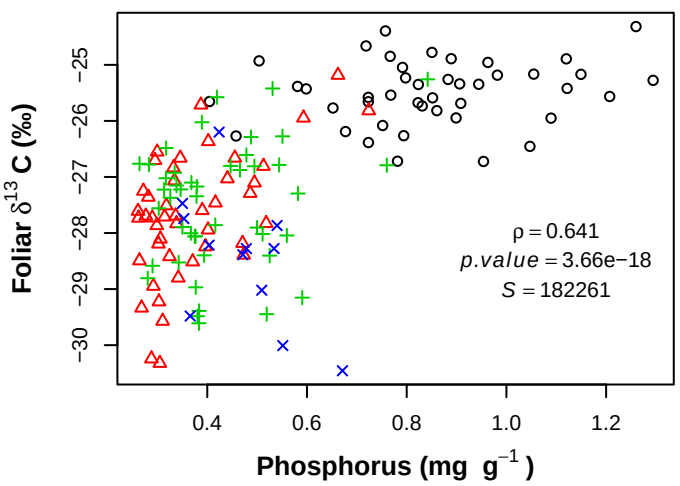

ρ = −0.59
p.value = 4.79e−15
S = 824554
Potassium (mg g^{-1})
Foliar δ^{18} O (‰)

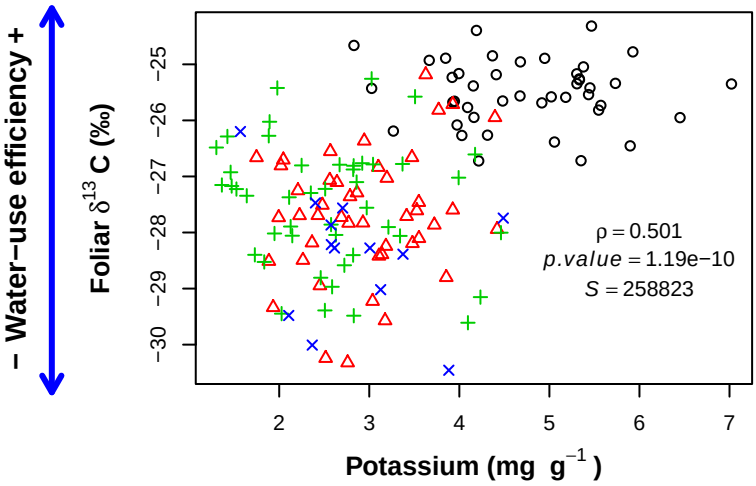

ρ = −0.622
p.value = 6.64e−17
S = 824202
Zinc (ppm)
Foliar δ^{18} O (‰)

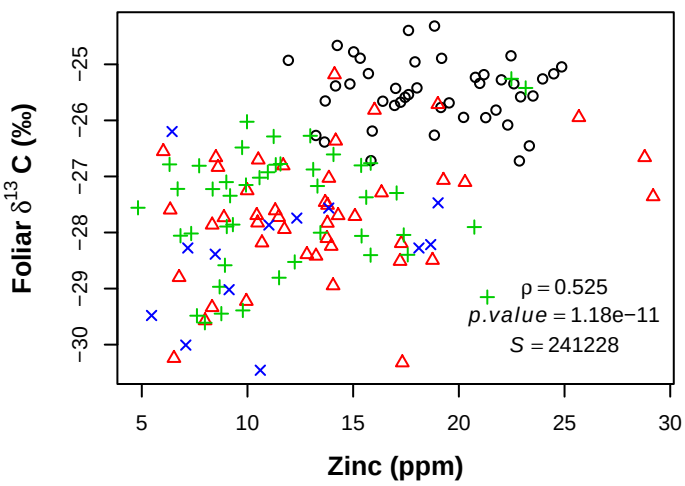

ρ = −0.696
p.value = 3.72e−22
S = 843888
Copper (ppm)
Foliar δ^{18} O (‰)
+
Stomatal conductance
−

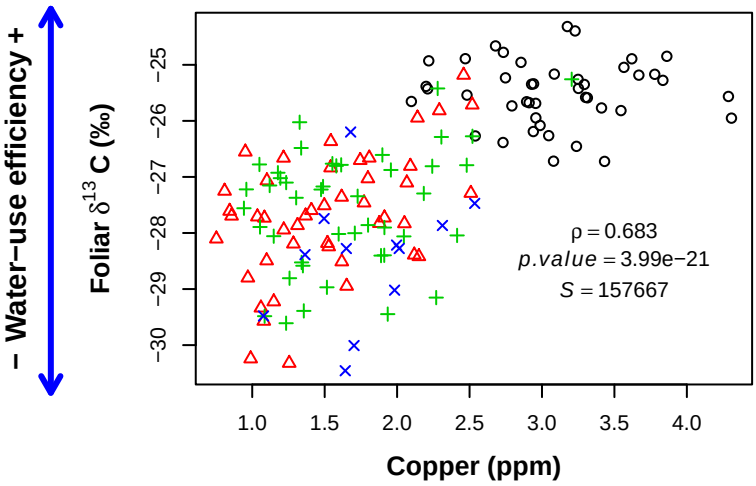

ρ = 0.764
p.value = 5.07e−28
S = 108006
N/P ratio
Foliar δ^{18} O (‰)
+
Stomatal conductance
−

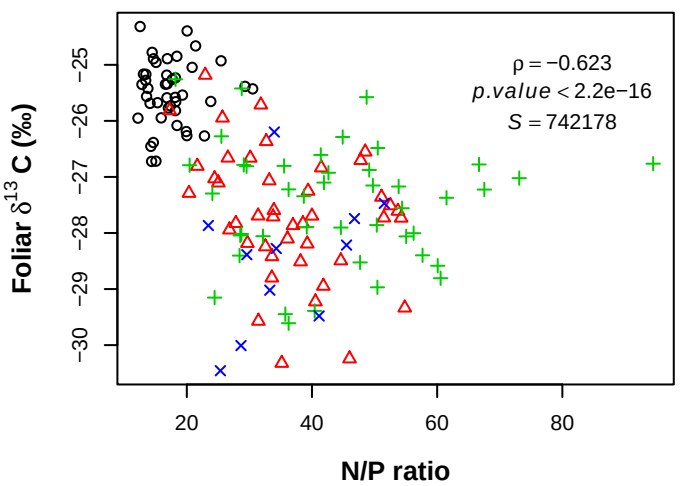

– Water–use efficiency +
Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
H N S U
ρ = 0.0367
p.value = 0.666
S = 450052
10
15
20
25
Nitrogen (mg g−1)

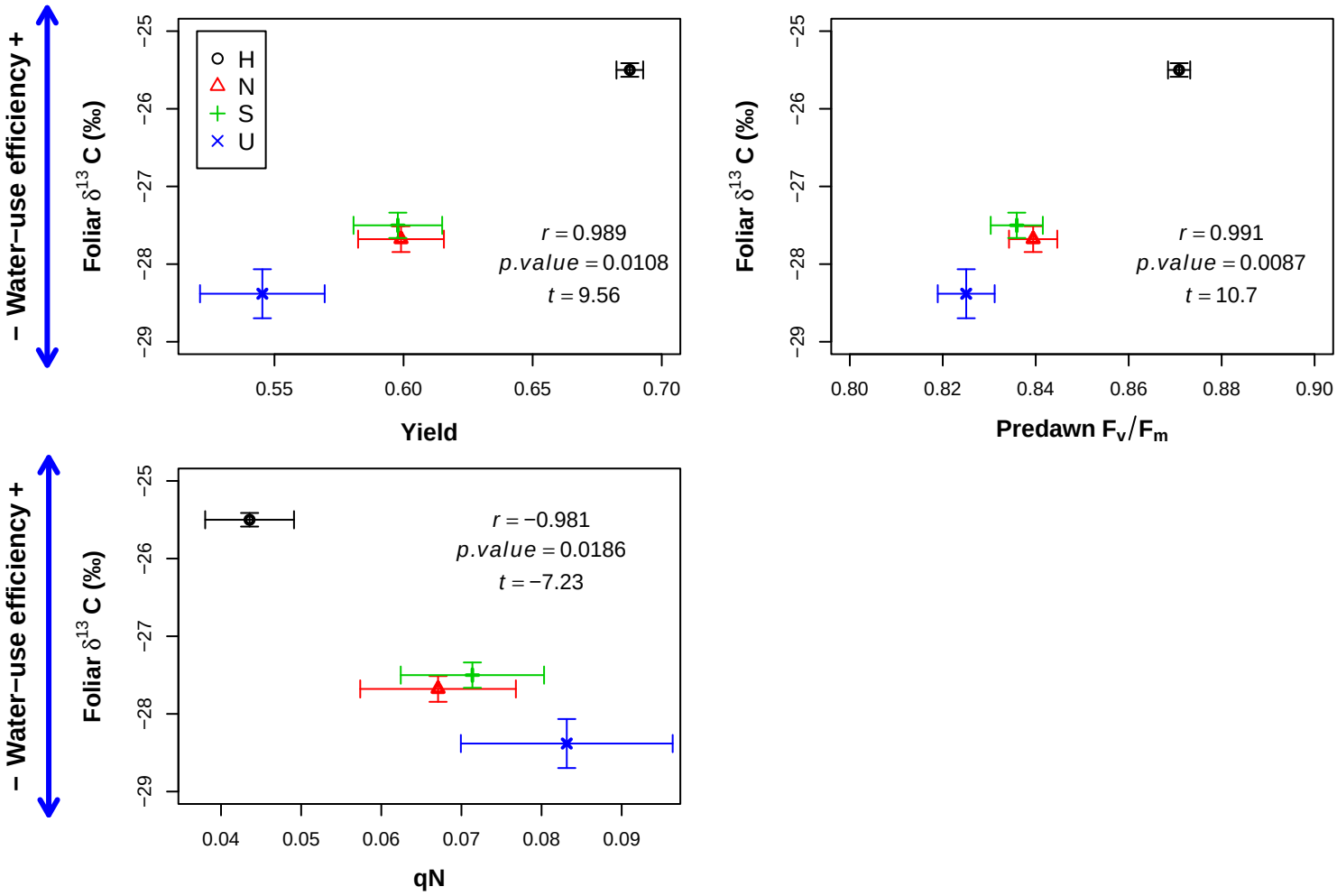

Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
ρ = 0.641
p.value = 3.66e−18
S = 182261
0.4
0.6
0.8
1.0
1.2
Phosphorus (mg g−1)

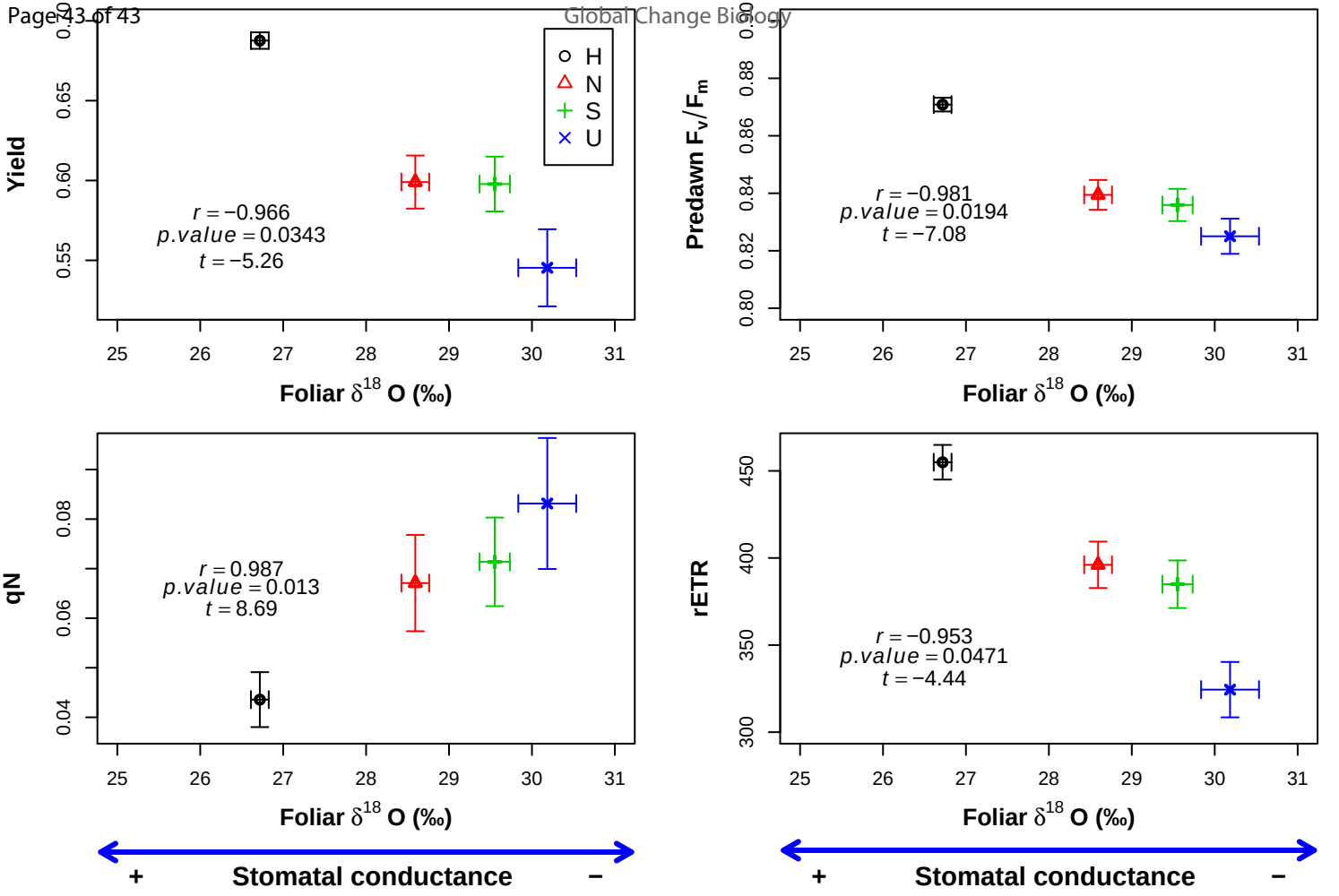

– Water–use efficiency +
Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
ρ = 0.501
p.value = 1.19e−10
S = 258823
2
3
4
5
6
7
Potassium (mg g−1)


Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
ρ = 0.525
p.value = 1.18e−11
S = 241228
5
10
15
20
25
30
Zinc (ppm)


– Water–use efficiency +
Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
ρ = 0.683
p.value = 3.99e−21
S = 157667
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Copper (ppm)


Foliar δ13 C (‰)
−25
−26
−27
−28
−29
−30
ρ = −0.623
p.value < 2.2e−16
S = 742178
20
40
60
80
N/P ratio


– Water−use efficiency +
Foliar δ13 C (‰)
−25
−26
−27
−28
−29
H
N
S
U
r = 0.989
p.value = 0.0108
t = 9.56
0.55
0.60
0.65
0.70
Yield
Foliar δ13 C (‰)
r = 0.991
p.value = 0.0087
t = 10.7
0.80
0.82
0.84
0.86
0.88
0.90
Predawn Fv/Fm
– Water−use efficiency +
Foliar δ13 C (‰)
r = −0.981
p.value = 0.0186
t = −7.23
0.04
0.05
0.06
0.07
0.08
0.09
qN


Yield
Predawn F_v/F_m
qN
rETR
Foliar δ^{18} O (‰)
H
N
S
U
r = −0.966
p.value = 0.0343
t = −5.26
r = −0.981
p.value = 0.0194
t = −7.08
r = 0.987
p.value = 0.013
t = 8.69
r = −0.953
p.value = 0.0471
t = −4.44
+
Stomatal conductance
−